LETTER TO JMG

Pregnancy does not influence colonic polyp multiplicity but may modulate upper gastrointestinal disease in patients with FAP

Nirosha Suraweera, Andrew Latchford, Amy McCart, Pauline Rogers, Sarah Spain, Oliver Sieber, Robin Phillips, Ian Tomlinson, Andrew Silver

J Med Genet 2007;**44**:541–544. doi: 10.1136/jmg.2007.049965

Background: Reproductive factors have been shown by epidemiology studies to alter colorectal cancer risk in women. Familial adenomatous polyposis (FAP) patients carry a germline adenomatous polyposis coli (APC) mutation predisposing to multiple adenoma formation in the intestine. The Min mouse provides a good model of FAP, and we recently reported a significant increase in intestinal tumour multiplicity in a recombinant line of mice following pregnancy.

Aim: We considered whether reproduction modulates intestinal tract disease in a large cohort of female patients with FAP (n = 180).

Results: Multiple regression analysis showed that the number of colonic polyps observed was not related to the person's pregnancy status nor the position of their APC germline mutation. The proportion of women attaining a high Spigelman stage (3 or 4) was unrelated to having a pregnancy prior to attaining the maximum Spigelman stage (p = 0.6). On the other hand, having a pregnancy significantly increased the proportion of women that attained the highest Spigelman stage when their APC germline mutation occurred within the mutation cluster region or at or after codon 1020 (50%, 6/12, p = 0.005 and 42%, 13/31, p = 0.006, respectively; multivariable logistic regression).

Conclusion: Our data suggest that reproduction may influence disease severity in the upper gastrointestinal tract in patients with FAP.

Patients with familial adenomatous polyposis (FAP) develop hundreds to thousands of colorectal polyps and, consequently, have an enhanced risk of colorectal cancer (CRC). Therapy consists of prophylactic colectomy, usually performed in the late teens or early 20s.[1 2] Adenomatous polyps also arise in the small intestinal tract, especially in the duodenum, and severity of disease can vary from no polyps to numerous lesions and carcinoma. Progressive disease is measured using a semi-quantitative assessment, referred to as the Spigelman stage.[3 4] patients with FAP carry a germline mutation in the *APC* gene, commonly within the β-catenin binding domains (codons 1020–1170 and 1265–2035). Furthermore, the position of the germline mutation determines the nature and location of the second hit.[5 6] If the mutation occurs within the mutation cluster region (MCR; codons 1194–1392), then there is strong selection for allelic loss of *APC* as the somatic mutation (''second hit'') in colorectal adenoma development.[7]

In the general population, men and women vary in CRC aetiology, and reproductive factors have been shown to alter CRC risk in women: parity has been inversely associated with colon cancer and use of hormone replacement therapy (HRT) decreases risk.[8–15] An analysis of the influence of pregnancy on both duodenal and colonic disease severity in female FAP has yet to be undertaken. However, we have recently shown that pregnancy leads to an increase in adenoma numbers in the small intestine of a recombinant line of Min mice. These mice carry a truncating mutation at codon 850 of the *APC* gene and are regarded as a good model of human FAP.[16] This observation provided impetus to considering pregnancy as a factor that might modulate intestinal disease severity in patients with FAP. Such a study is necessary because a significant proportion of female patients have completed a pregnancy before colectomy, and fecundity in patients with FAP is close to that of the general population.[17] The extensive database of patients with FAP at St Mark's Hospital, which includes a unique collection of accurate counts of polyp numbers in the colon, provided an ideal opportunity to undertake a detailed analysis on the influence of pregnancy on both duodenal and colonic disease severity in female patients with FAP.

MATERIALS AND METHODS

Archive of patients with FAP

For this study, information was available from the polyposis registry at St Mark's Hospital for 180 female patients from 133 families, including 13 sibling pairs, 2×4 siblings, 4×3 siblings and 1×5 siblings. All had undergone a colectomy and side-viewing gastroduodenoscopy to assess Spigelman stage.[4] The number of pregnancies resulting in a live birth and the age at the time of recording the maximum Spigelman stage was established for each patient. Age at colectomy, along with accurate colonic polyp counts on colectomy specimens (performed as described previously[18]) was known for 72.2% (130/180) of patients; the number of pregnancies prior to colectomy was also recorded for these women. The usual practice at St Mark's is for the decision to perform colectomy to be tailored to an individual's wishes and needs; age at colectomy does not appear to influence polyp number.[18] In addition, the patient's germline *APC* mutation was known for 71% (92/130) of patients with colonic polyp counts; the age distribution for this subset of cases did not differ significantly from the full dataset. Maximum Spigelman stage and germline data were available for 68% (123/180) patients. Again, the age distribution for this subset of cases did not differ significantly from the full dataset. Germline data were not available for cases that had not been screened or screened fully for APC germline mutations (32% 57/180). We also calculated patient survival by the time difference between the date of colectomy or date of attaining maximum

Abbreviations: APC, adenomatous polyposis coli; CRC, colorectal cancer; FAP, familial adenomatous polyposis; HRT, hormone replacement therapy; MCR, mutation cluster region

Table 1 Colonic polyp counts at colectomy associated with germline APC mutation and pregnancy status of female patients with FAP

Germline mutation	Pregnancy before colectomy	No of patients (median polyp count)	IQR	p Value *	Germline mutation	Pregnancy before colectomy	No of patients (median polyp count)	IQR	p Value *
Non-MCR	No	42 (500)	285 to 955	0.8	<1020aa	No	21 (800)	500 to 1000	0.6
	Yes	33 (870)	200 to 1200			Yes	22 (744)	100 to 1357	
MCR	No	12 (2625)	1000 to 3500	0.04	⩾1020aa	No	33 (854)	300 to 1293	0.6
	Yes	5 (800)	750 to 992			Yes	16 (955)	625 to 1129	

aa, amino acid; IQR, interquartile range.
*p Values are from testing for a significant difference in the number of polyps between women who experienced a pregnancy before colectomy and those who did not, nested within categories of germline mutation. Multiple linear regressions adjusted for age at colectomy and clustered on family.

Spigleman scores to the last examination date, and included this in the multiple regression analysis.

Statistical analysis

Data were analysed using Stata V.8.2 (Stata statistical software release 8.0; Stata Corp., College Station, Texas, USA). The data were analysed using multiple logistic regression for the outcome measure of proportion of cases attaining a Spigelman stage of 3 or 4 and multiple linear regression for polyp counts at colectomy. Regressions investigated whether the outcomes varied with pregnancy, number of pregnancies or germline mutation, and were adjusted for age at maximum Spigelman stage/colectomy of patient. Age was grouped as <20, 20–29, 30–39, 40–49 and ⩾50 years. As the dataset included women from the same family, all regressions were clustered on family and this was not a significant confounding factor in our regression analysis.

In patients for whom the number of colonic polyps at colectomy was known, a wide range of values was recorded and the distribution was very skewed. The data were normalised by taking the fourth root transformation of polyp numbers. Multiple regression analysis was performed by two methods (1) with the fourth root of polyp number for an individual as the outcome and (2) with the number of polyps ranked from the smallest to the largest over the series. There was no difference in results from the two regressions, and so only results from the fourth-root analyses are presented.

Germline mutations were analysed using two methods of multiple testing: (1) the germline mutations were grouped as MCR (codons 1250–1400) and non-MCR (all other codons) and (2) they were ranked from 5′ to 3′ of codon 163–1925 and two mutation groups were created: codon <1020 and ⩾1020. Bonferroni correction was applied to the germline mutation variable to allow for multiple testing: MCR and <1020 reached statistical significance at $p = 0.03$. The codon ⩾1020 mutation group included all beta-catenin binding sites within the *APC* gene.

RESULTS

We first considered whether pregnancy influenced the number of polyps detected at colectomy. In total, 130 patients with FAP had accurate colonic polyp counts, and 63% (82/130) were identified as having completed at least one pregnancy before surgery. After adjusting for age at colectomy, multiple regressions showed that polyp number did not vary between parous and non-parous women. Similarly, the number of pregnancies before colectomy did not influence polyp numbers. In addition, after adjusting for age, survival of the patients after colectomy was not significantly affected by pregnancy. Analysis of archival data on the severity of upper gastrointestinal disease in women with FAP ($n = 180$; 61%, 109/180, completed at least one pregnancy) revealed, as expected, that the proportion of cases attaining a high Spigelman stage (3 or 4) increased significantly with age (age <20, 1/5, 20%; >50, 17/37, 46%) and was unrelated to having a pregnancy prior to attaining the maximum stage ($p = 0.6$, logistic regression). After adjusting for patient age, the proportion of cases reaching a high stage was not influenced by a single pregnancy or multiple pregnancies ($p = 1.0$; logistic regression). In addition, after adjusting for patent age, survival of a patient after the maximum Spigelman score is not affected by pregnancy.

A consistent genotype–phenotype correlation exists in FAP, based on the position of the germline mutation, the non-random nature of the subsequent somatic mutations and the linked selection for a particular number of β-catenin degradation repeats.[4–6] Consequently, we analysed the subset of patients for whom the germline mutation and colonic polyp counts were known (71%, 92/130). Patients were grouped as having a germline mutation within the MCR or a mutation outside this region (non-MCR). We then nested, within each mutation group, women who had completed a pregnancy and others who had not. Multiple logistic regressions adjusted for age and cluster on family showed that pregnancy before colectomy did not affect polyp counts in the non-MCR group ($p = 0.8$). In contrast, there was a borderline significant

Table 2 Maximum Spigelman stage associated with germline APC mutation and pregnancy status of female patients with familial adenomatous polyposis

Germline mutation	Pregnancy before highest stage	n (%)		p Value†	Germline mutation	Pregnancy before highest stage	n (%)		p Value†
		Stage 1/2*	Stage 3/4*				Stage 1/2*	Stage 3/4 *	
Non-MCR	No	29 (78)	8 (22)	0.3	<1020	No	12 (75)	4 (25)	0.9
	Yes	44 (72)	17 (28)			Yes	32 (76)	10 (24)	
MCR	No	13 (100)	0 (0)	<0.01	⩾1020	No	30 (88)	4 (12)	<0.01
	Yes	6 (50)	6 (50)			Yes	19 (58)	13 (42)	

*n (%) with maximum Spigelman stage.
†pValues are from testing for a significant difference in the proportion of women who experienced a high Spigelman stage before pregnancy and those who did not, nested within categories of germline mutation (MCR vs non-MCR and before vs after nucleotide 1020. Logistic regression adjusted for age at colectomy and clustered on family.

(p = 0.04) difference within the MCR germline mutation group between the pregnancy and non-pregnancy subgroups (table 1). However, when patients were divided into those with germline mutation before codon 1020 (removing all β-catenin degradation repeats in the mutant germline allele) and those with a mutation at or after codon 1020, then the number of colonic polyps was not significantly affected by pregnancy before colectomy in either group (p = 0.6; table 1).

For Spigelman stage, we found, using logistic regression adjusted for age and clustered on families, that within the MCR group there was a significantly higher proportion of parous women with a high stage (50%, 6/12) than among women who had not experienced a pregnancy (0%, 0/13; p = 0.005, logistic regression; table 2). This significant difference was retained when patients were grouped according to position relative to codon 1020; significantly, more women carrying a ⩾1020 germline mutation who had experienced a pregnancy attained a high Spigelman stage (42%, 13/31 p = 0.006) than women who had not reproduced (12%, 4/34). Furthermore, logistic regression analysis, adjusted for age, indicated that the proportion of women with a high Spigelman stage (3/4) increased with respect to the number of pregnancies, but only in the MCR mutation group (OR = 3.7, 95% CI 0.9 to 15.2, p = 0.07).

DISCUSSION

As standard therapy in FAP usually consists of restorative proctocolectomy or colectomy with an ileorectal anastomosis, it is essential that the clinician is aware of risk factors, genetic or environmental, associated with subsequent adenomatous polyp formation in the remaining small intestine. Fecundity in female patients with FAP is close to that of the general population[17] and in our study 39% (71/180) women had completed a pregnancy before colectomy. Thus, advice on reproductive matters needs to be given to women with FAP, including contraception and pregnancy (which is known to influence desmoid disease) and any associated risk of polyp formation in the remaining small intestine and CRC. Analogous to extensive investigations in breast cancer, it is very likely that local steroid metabolism in the colon plays a key role in modulating the effects of hormones such as oestrogen. There may be variation between men and women in CRC aetiology by subsite.[19 20] For example, the incidence of cancer of the caecum and ascending colon is higher in women than men; such sex-specific differences in colon cancer have been attributed to oestrogen,[21] and/or the effects on bile acid metabolism secondary to hormonal changes.[21–24]

Key points

- We reported recently that a recombinant line of Min mice (a mouse model of familial adenomatous poyposis, FAP) showed increased tumour multiplicity after pregnancy. In this article, we considered whether reproduction modulates intestinal tract disease in women with FAP.
- Colonic polyp counts at colectomy were not influenced by prior pregnancy, but pregnancy significantly increased the proportion of patients that attained the highest Spigelman stage when their germline mutation occurred within the MCR region, or at or after codon 1020.
- Results indicated that reproduction may influence disease severity in the upper gastrointestinal tract in patients with FAP.

The Min mouse is a good model of FAP and we have reported recently on two recombinant inbred lines (I and V) and the location of a modifier (*Mom3*) close to *Apc* that alters adenoma numbers by modifying the frequency of wild-type allele loss at *Apc*; line V had more severe disease and higher rates of loss in tumours.[25] Subsequently, we have shown that only line I is susceptible to pregnancy-associated adenoma formation and that this is under genetic control. The major environmental factor, namely diet, was the same for both lines V and I. We observed a statistically significant increase in the frequency of loss of wild-type allele in parous line 1 mice relative to virgin controls.[16] These observations raised the distinct possibility of pregnancy causing more severe disease in female patients with FAP. Our analysis of colonic polyp number and Spigelman stage at colectomy in women with FAP identified several intriguing possibilities with regard to the influence of pregnancy. We observed that women with a germline mutation in the MCR region of *APC* who had had a pregnancy tended to have a significantly higher Spigelman stage than mutation-equivalent women that had not been pregnant. Furthermore, mothers with a mutation at or after codon 1020 also had a higher Spigelman stage compared with those who were not pregnant. Although not significant, there is also a suggestion that the number of pregnancies influences the proportion of women with a higher Spigelman stage; this needs to be tested in a larger independent set of samples. This would suggest a potential deleterious effect of pregnancy in patients with FAP with this type of germline mutation (MCR mutations or codon ⩾1020). The codon ⩾1020 region contains all the β-catenin binding domains and the MCR region.

Clearly, the findings presented here will need to be confirmed in an independent dataset. It should also be acknowledged that this study does not account for several other factors that have the potential to influence colonic polyposis or Spigelman stage, including use of hormonal oral contraceptives or HRT, duration of breastfeeding, miscarriages and termination of pregnancy. Nonetheless, the results on patients with FAP and on Min mice do implicate hormonal effects as a potential important aspect of intestinal tumorigenesis in women. The precise mechanisms by which hormones such as oestrogens influence susceptibility to CRC remain to be elucidated.

ACKNOWLEDGEMENTS

We thank Jackie Wright, Sandra Burke and Kay Neale of the St Mark's Polyposis registry.

Authors' affiliations

N Suraweera, A McCart, A Silver, ICMS, Barts and The London Queen Mary's School of Medicine and Dentistry, London, UK
P Rogers, Cancer Research UK Colorectal Cancer Unit, St Mark's Hospital, Harrow, Middlesex, UK
A Latchford, R Phillips, A Silver, Cancer Research UK Colorectal Cancer Unit and Polyposis Registry, St Mark's Hospital, Harrow, Middlesex, UK
S Spain, O Sieber, Molecular and Population Genetics Laboratory, London Research Institute, Cancer Research UK, London, UK
I Tomlinson, Molecular and Population Genetics Laboratory, London Research Institute, Cancer Research UK, London, UK, and Cancer Research UK Colorectal Cancer Unit, St Mark's Hospital, Harrow, Middlesex, UK

Competing interests: None declared.

Correspondence to: Professor Andrew Silver, Institute of Cell and Molecular Science, Academic Surgery, Barts and The London, Queen Mary's School of Medicine and Dentistry, 4 Newark Street, London, E1 2AT; a.r.silver@qmul.ac.uk

Received 26 February 2007
Revised 24 April 2007
Accepted 25 April 2007
Published Online First 11 May 2007

REFERENCES

1 **Boyle P**, Langman JS. ABC of colorectal cancer. Epidemiology. *BMJ* 2000;**8**:452–5.
2 **Cannon-Albright LA**, Skolnick MH, Bishop DT, Lee RG, Burt RW. Common inheritance of susceptibility to colonic adenomatous polyps and associated colorectal cancers. *N Engl J Med* 1988;**319**:533–7.
3 **Lichtenstein P**, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A, Hemminki K. Environmental and heritable factors in the causation of cancer. *N Engl J Med* 2000;**343**:78–85.
4 **Spigelman AD**, Williams CB, Talbot IC, Domizio P, Phillips RK. Upper gastrointestinal cancer in patients with familial adenomatous polyposis. *Lancet* 1989;**2**:783–5.
5 **Lamlum H**, Ilyas M, Rowan A, Clark S, Johnson V, Bell J, Frayling I, Efstathiou J, Pack K, Payne S, Roylance R, Gorman P, Sheer D, Neale K, Phillips R, Talbot I, Bodmer W, Tomlinson I. The type of somatic mutation at APC in familial adenomatous polyposis is determined by the site of the germline mutation: a new facet to Knudson's 'two-hit' hypothesis. *Nat Med* 1999;**5**:1071–5.
6 **Rubinfeld B**, Souza B, Albert I, Muller O, Chamberlain SH, Masiarz FR, Munemitsu S, Polakis P. Association of the APC gene product with beta-catenin. *Science* 1993;**262**:1731–4.
7 **Fearnhead NS**, Britton MP, Bodmer WF. The ABC of APC. *Hum Mol Genet* 2001;**10**:721–33.
8 **Johns LE**, Houlston R. A systematic review and meta-analysis of familial colorectal cancer risk. *Am J Gastroenterol* 2001;**96**:2992–3003.
9 **Fraumeni JF Jr**, Lloyd JW, Smith EM, Wagoner JK. Cancer mortality among nuns: role of martial status in etiology of neoplastic disease in women. *J Natl Cancer Inst* 1969;**42**:455–68.
10 **Weiss NS**, Daling JR, Chow WH. Incidence of cancer of the large bowel in women in relation to reproductive and hormonal factors. *J Natl Cancer Inst* 1981;**67**:57–60.
11 **Kune GA**, Kune S, Watson LF. Children, age at first birth, and colorectal cancer risk. Data from the Melbourne Colorectal Cancer Study. *Am J Epidemiol* 1989;**129**:533–42.
12 **Bostick RM**, Potter JD, Kushi LH, Sellers TA, Steinmetz KA, McKenzie DR, Gapstur SM, Folsom AR. Sugar, meat, and fat intake, and non-dietary risk factors for colon cancer incidence in Iowa women (United States). *Cancer Causes Control* 1994;**5**:38–52.
13 **Potter JD**. Hormones and colon cancer. *J Natl Cancer Inst* 1995;**87**:1039–40.
14 **Herbert-Croteau N**. A meta-analysis of hormone replacement therapy and colon cancer in women. *Cancer Epidemiol Biomark Prev* 1998;**7**:653–60.
15 **Kampman E**, Potter JD, Slattery ML, Caan BJ, Edwards S. Hormone replacement therapy, reproductive history, and colon cancer: a U.S. multi-center case-control study. *Cancer Causes Control* 1997;**8**:146–58.
16 **Suraweera N**, Haines J, McCart A, Rogers P, Latchford A, Coster M, Polanco-Echeverry G, Guenther T, Wang J, Sieber O, Tomlinson I, Silver A. Genetic determinants modulate susceptibility to pregnancy-associated tumourigenesis in a recombinant line of Min mice. *Hum Mol Gen* 2006;**15**:3429–35.
17 **Church JM**, McGannon E. Prior pregnancy ameliorates the course of intra-abdominal desmoid tumors in patients with familial adenomatous polyposis. *Dis Colon Rectum* 2000;**43**:445–50.
18 **Crabtree M**, Tomlinson I, Talbot I, Phillips RK. Variability in the severity of colonic polyposis in FAP results from differences in tumour initiation rather than progression and depends relatively little on patient age. *Gut* 2001;**49**:540–3.
19 **Martinez ME**, Grodstein F, Giovannucci E, Colditz GA, Speizer FE, Hennekens C, Rosner B, Willett WC, Stampfer MJ. A prospective study of reproductive factors, oral contraceptive use, and risk of colorectal cancer. *Cancer Epidemiol Biomarker Prev* 1997;**6**:1–5.
20 **Czarnomska A**, Krysiak E, Piskorowska J, Sitarz M, Pysniak K, Pilcik T, Demant P. Opposite effects of modifiers of the Apc^{Min} mutation in intestine and mammary gland. *Cancer Res* 2003;**63**:4533–7.
21 **Yoo KY**, Tajima K, Inoue M, Takezaki T, Hirose K, Hamajima N, Park SK, Kang DH, Kato T, Hirai T. Reproductive factors related to the risk of colorectal cancer by subsite: a case-control analysis. *Br J Cancer* 1999;**79**:1901–6.
22 **Bufill JA**. Colorectal cancer: evidence for distinct genetic categories based on proximal or distal tumor location. *Ann Intern Med* 1990;**1134**:779–88.
23 **McMichael AJ**, Potter JD. Reproduction, endogenous and exongenous sex hormones, and colon cancer. A review and hypothesis. *J Natl Cancer Inst* 1980;**65**:1201–7.
24 **McMichael AJ**, Potter JD. Do intrinsic sex differences in lower alimentary tract physiology influence the sex-specific risks of bowel cancer and other biliary and intestinal disease? *Am J Epidemiol* 1983;**118**:620–7.
25 **Haines J**, Johnson V, Pack K, Suraweera N, Slijepcevic P, Cabuy E, Coster M, Ilyas M, Wilding J, Sieber O, Bodmer W, Tomlinson I, Silver A. Genetic basis of variation in adenoma multiplicity in $Apc^{Min/+}$ $Mom1^{S}$ mice. *Proc Natl Acad Sci U S A* 2005;**102**:2868–73.

Correction

doi: 10.1136/jmg.2006.045765corr1

Zhang Y, Malekpour M, Al-Madani N, Kahrizi K, Zanganeh M, Mohseni M, Mojahedi F, Daneshi A, Najmabadi H, Smith RJH. Sensorineural deafness and male infertility: a contiguous gene deletion syndrome. *J Med Genet* 2007;**44**:233–240.

A co-author was inadvertently omitted (Naomi J Lohr; Department of Otolaryngology, University of Iowa, Iowa City, Iowa, USA). The authors should be as follows: Zhang Y, Malekpour M, Al-Madani N, Kahrizi K, Zanganeh M, Lohr NJ, Mohseni M, Mojahedi F, Daneshi A, Najmabadi H, Smith RJH.